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ON VARIATIONS IN THE PHAGOCYTIC AND COCCIDAL POWER OF THE BLOOD IN PNEUMONIA AND SCARLET FEVER.*

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It has been shown by various observers that the phagocytic activity of leukocytes may vary just as the opsonic power of the serum varies, and that it is necessary to examine both leukocytes and serum if one wishes to determine so far as possible the actual capacity of the blood for phagocytosis.

Rosenow¹ found that the leukocytes from pneumonia patients were perceptibly more actively phagocytic than normal leukocytes, as were also leukocytes from patients with puerperal sepsis and appendicitis. He points out that this is possibly due to the preponderance of young leukocytes. This increased phagocytic power of pneumonic leukocytes seemed to be without reference to the stage of the disease. Potter and Krumwiede² found that at the height of streptococcus and pneumococcus infections the phagocytic power of the leukocytes as compared with that of leukocytes of supposedly normal persons was diminished, and more noticeably so than the opsonic index of the serum, but that during recovery the phagocytic power rose well above the normal, and again distanced the opsonic index; during convalescence the phagocytic power of the leukocytes and the opsonic index gradually fell to normal or just below. They made similar observations in staphylococcus infections and tuberculosis. Shattock and Dudgeon³ observed that washed pneumonic leukocytes were more active than washed normal leukocytes in the serum of three different patients (empyema, urinary fever, and pneumonia), the difference being most marked with pneumonic serum. Achard, Ramond, and Foix⁴ found that the activity of the leukocytes and the opsonic

* Received for publication December 17, 1910.

¹ *Jour. Infect. Dis.*, 1906, 3, p. 683.

² *Jour. Am. Med. Assn.*, 1907, 49, p. 1815; *Jour. Infect. Dis.*, 1907, 4, p. 601.

³ *Proc. Royal Soc.*, 1907, B, 80, p. 165.

⁴ *Compt. rend. soc. biol.*, 1909, 66, p. 1031.

index, especially with reference to yeast cells, followed the same course in the acute infectious diseases (typhoid fever, pneumonia, scarlet fever, erysipelas, tuberculosis, and meningitis), being diminished at first and later increasing, except in cases of death, when both would be depressed. They obtained more striking results with yeast cells than with bacteria.

Rosenow¹ has studied also the changes that occur in the phagocytic activity of the leukocytes in cases of chronic or subacute pneumococcus and staphylococcus endocarditis; he found that increased phagocytic activity was not associated necessarily with an increase in the power to destroy the infecting bacteria.

In a case of recurrent and complicated erysipelas, Boughton² observed a specificity in the relation of serum, leukocytes, and bacteria to each other. He calls attention to the fact that so far these specific variations of serum and leukocytes with respect to the invading bacterium have been observed only in cases of chronic infection. He found that variations in the opsonic power of the serum might occur independently of variations of the phagocytic power of the leukocytes, but that such variations on the part of the leukocytes were not entirely independent of the variations of the serum. The changes in the serum appeared earlier and were more persistent and more marked than the changes in the leukocytes. He considered that the low phagocytic power of the leukocytes in their own serum might depend either upon the lack of some element necessary to phagocytosis or to some inhibitory substance specific for the leukocytes in question and neutralized by normal serum.

It has been shown that human serum has no bactericidal action on streptococci (Weaver and Ruediger,³ Ruediger,⁴ Meakins⁵) and that washed leukocytes alone have no bactericidal power (Ruediger), but Ruediger found that when leukocytes were added to the serum, the streptococci were destroyed. In an attack of post-scarlatinal nephritis, Ruediger observed that not only was the opsonin diminished but also the leukocytes had lost some of

¹ *Jour. Infect. Dis.*, 1909, 6, p. 245.

² *Ibid.*, 1910, 7, p. 111.

³ *Medicine*, 1903, 9, p. 515; *Trans. Chi. Path. Soc.*, 1903, 5, p. 285.

⁴ *Jour. Amer. Med. Assn.*, 1906, 46, p. 108.

⁵ *Jour. Exp. Med.*, 1908, 11, p. 100.

their power to destroy streptococci. Rosenow¹ has demonstrated that neither normal (human) nor pneumonic serum has any pneumocidal action, the pneumocidal action of pneumonic or other leukocytic blood being the result of the combined action of the serum and leukocytes.

The following experiments have been made to determine (1) the changes, if any, in the phagocytic activity of the leukocytes during the course of pneumonia and scarlet fever; (2) the specificity or non-specificity of the changes in phagocytic activity; and (3) the relation between the phagocytic and destructive power of the leukocytes. Similar experiments were carried out also in a case of chronic rheumatism receiving injections of galactose-killed streptococci.

It was necessary to use sterile blood on account of the coccidial experiments. Hence sterilized tubes and solutions were used in collecting the blood from the ear, the lobe being first washed carefully with alcohol. There was rarely any contamination. Normal human serum and leukocytes were used as controls. In order to compare the phagocytic activity of the leukocytes of a patient with that of the leukocytes of a normal person, it is essential to use suspensions containing the same number of polymorphonuclear leukocytes. To obtain such suspensions, the number of polymorphonuclear leukocytes in each suspension is counted by means of a white blood counting apparatus and the suspensions are equalized by the addition of the required amount of normal salt solution.

The opsonic estimations were made in the usual way, using equal parts of serum, leukocytes, and bacterial suspension. The experiments were controlled from time to time by diluting the serum to the point of opsonic extinction. In this case both normal and patient's sera were first heated at 60° C. for 10 minutes. As in previous experiments of this kind the two methods were found now also to yield the same results.

In the regular routine, four specimens are prepared for each organism, namely, from mixtures of normal serum with normal leukocytes and with patient's leukocytes, and of patient's serum with normal leukocytes and patient's leukocytes. From the study

¹ *Jour. Infect. Dis.*, 1906, 3, p. 683.

of these specimens one can determine: (1) the opsonic index with normal and with patient's leukocytes; (2) the phagocytic activity of the patient's leukocytes (cytophagic index of Glynn and Cox¹) by determining the relation of the phagocytic index of mixtures of normal serum and patient's leukocytes to that of mixtures of normal serum and normal leukocytes; (3) the phagocytic power of the whole blood (opsono-cytophagic index of Glynn and Cox), obtained by determining the relation between the phagocytic index of a mixture of the patient's serum and the patient's leukocytes and that of a mixture of normal serum and normal leukocytes.

Exactly the same mixtures are made to determine the destructive power of the patient's serum and leukocytes and of normal serum and normal leukocytes. In all cases accurately standardized suspensions of leukocytes must be used. For these experiments a very thin bacterial suspension is necessary, the suspension used for opsonic determinations being diluted about 30 times. Suspensions of leukocytes thicker than those used in opsonic work are required, an average of 20,000 leukocytes per cubic millimeter being sufficient. The mixtures of equal parts of serum, bacterial suspension, and standardized leukocytic suspension are incubated 18 hours, when a loopful of each mixture is plated, defibrinated blood (goat) being added to each tube of melted agar to more easily distinguish the colonies (see Fig. 1). The number of colonies is counted at the end of 24 hours' incubation at 37° C. By dividing the number of colonies on the plate made from the mixture containing normal serum and normal leukocytes by the number on the plate made from the mixture containing patient's serum and normal leukocytes, one gets a figure representing the destructive power of the patient's serum as compared with that of normal serum, normal leukocytes being used in both cases. This figure corresponds, then, to the opsonic index. By dividing the number of colonies on the plate made from a mixture of normal serum and leukocytes by the number of colonies on the plate made from the mixture containing normal serum and patient's leukocytes, one obtains a figure which represents the destructive power of the patient's leukocytes as compared with normal leukocytes, normal

¹ *Jour. Path. and Bact.*, 1909, 14, p. 90.

serum being used in each case. This figure corresponds to the cytophagic index. By again dividing the number of colonies on the plate from the mixture of normal serum and normal leukocytes by the number on the plate from the mixture of the patient's serum and patient's leukocytes, one obtains a figure representing the destructive power of the whole blood of the patient as compared with that of normal blood. This figure corresponds to the opsonocytophagic index.

Where there is much phagocytosis of red blood cells, as occurs with certain normal sera and certain pneumonic and scarlatinal leukocytes, the number of bacteria engulfed may be so diminished that correct phagocytic determinations are impossible.

In the case of pneumonia, opsonic estimations were made with the pneumococcus, streptococcus, and staphylococcus. Bactericidal experiments were made with the pneumococcus only. The pneumococcus employed was one cultivated from the blood of a pneumonia patient. A non-virulent strain is necessary, one which is readily phagocytizable, especially for the bactericidal experiments. The staphylococcus was isolated from a furuncle and the streptococcus was a typical *Streptococcus pyogenes*.

The opsonic determinations in the case of the scarlet fever patients were made with the same streptococcus. In the majority of cases the homologous streptococcus and in some mixed cases the homologous diphtheria bacillus were also employed. The bactericidal experiments were made with streptococcus; in one case only was the homologous streptococcus also used. The results corresponded closely, whatever the streptococcus strain used (Chart 2).

In the case of chronic rheumatism the early opsonic estimations were made with the pneumococcus, staphylococcus, and homologous streptococcus, isolated from the excised tonsil by Dr. D. J. Davis. Later the streptococcus only was used. The bactericidal experiments were all made with the homologous streptococcus.

Nine cases of pneumonia in adult men were studied. No examinations were made before the fourth day of the disease because the patients did not enter the hospital earlier. Both before and after crisis the opsonic power of the serum was found to be normal in all of the mild cases except one, where it was slightly increased

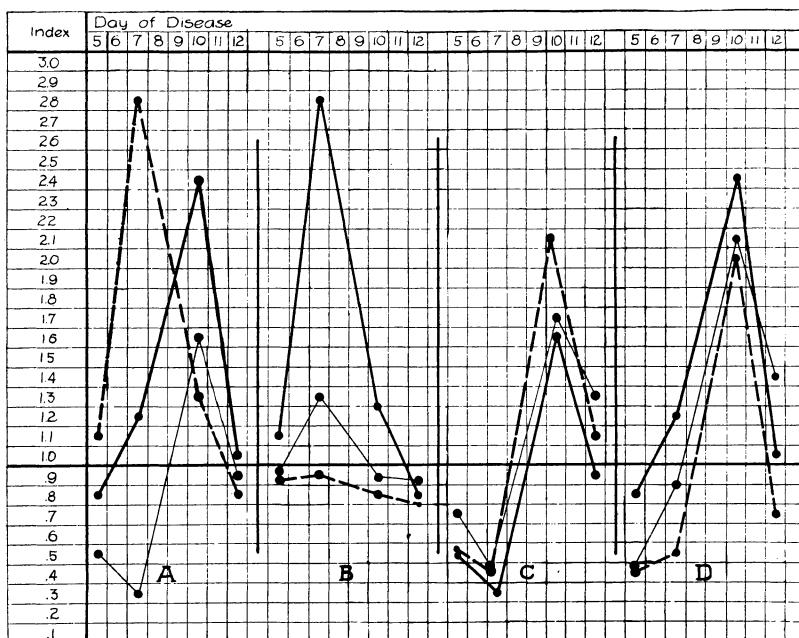


CHART 1.—Severe case of pneumonia (adult man).

A.

Heavy broken line = The pneumococcus opsonic power of patient's serum compared with that of normal serum (opsonic index).

Fine solid line = The phagocytic power of patient's leukocytes with respect to pneumococcus compared with that of normal leukocytes under the influence of normal serum (cytophagic index).

Heavy solid line = The phagocytic power of the patient's whole blood with respect to pneumococcus compared with that of normal blood (opsono-cytophagic index).

B.

The Opsonic Power of the Patient's Serum Compared with Normal Serum.

Heavy dotted line = The pneumococcus opsonic index.

Heavy dotted line = The pneumococcus opsonic index
 Fine solid line = The staphylococcus opsonic index

Heavy solid line = The staphylococcus opsonic index.
Heavy broken line = The streptococcus opsonic index.

6

The Phagocytic Power of Patient's Leukocytes Compared with That of Normal Leukocytes under the Influence of Normal Serum.

Heavy solid line = The pneumococcus cytophagic index.

Fine solid line = The staphylococcus cytophagic index.

Thin solid line = The staphylococcus cytophagic index.
 Heavy broken line = The streptococcus cytophagic index

D

The Phagocytic Power of the Patient's Whole Blood Compared with That of Normal Blood (Opsono-cytophagic Index).

Heavy solid line = The pneumococcus opsono-cytophagic index

Heavy solid line = The pneumococcus opsono-cytolytic
Fine solid line = The staphylococcus opsono-cytolytic in

Heavy solid line = The staphylococcus opsono-cytolytic index.
Heavy broken line = The streptococcus opsono-cytolytic index.

on the 15th day of the disease. In one fatal case examined before crisis, the opsonin also was normal. In another severe case (Chart 1-B) the opsonic power was normal early in the disease, but with improvement in the patient's condition it increased. In two other severe cases the opsonic power of the serum was below normal before crisis, but rose considerably afterward.

In these cases of pneumonia the phagocytic power of the leukocytes (cytrophagic index) was also estimated. In the mild cases, both before and after crisis, it was found to be greater than normal, except once, in those cases in which only one estimation was made on the same patient. In one patient examined both before and after crisis, the phagocytic power of the leukocytes was found to be below normal before crisis and increased afterward. One fatal case showed normally active leukocytes. The other severe cases all showed diminished leukocytic activity during the height of the disease with an increased power as the patient improved (Chart 1-C).

Pneumococidal experiments were made to determine if the destructive and phagocytic power corresponded. It was found that in the majority of experiments, increased or decreased phagocytosis meant increased or decreased destructive power.

The cases of pneumonia examined showed then specific variations in the opsonic power of the serum for the pneumococcus (Chart 1-B) but no specific variations in the phagocytic power of the leukocytes (Chart 1-C) or in the phagocytic power of the whole blood (opsonocytrophagic index, Chart 1-D), the leukocytes showing the same variations in phagocytic activity when tested with the streptococcus and the staphylococcus as with the pneumococcus.

Thirteen cases of scarlet fever were studied in the same way. Four were mild, one moderately severe, and eight severe, three of these being fatal. In the four mild, uncomplicated cases, there was little or no change in the phagocytic activity of the leukocytes with respect to the streptococcus, staphylococcus, and pneumococcus, although the opsonic index was low at first, followed by a rise for the streptococcus as improvement occurred.

In the moderately severe case, after a slight rise, the opsonic index remained persistently low. The leukocytes at no time be-

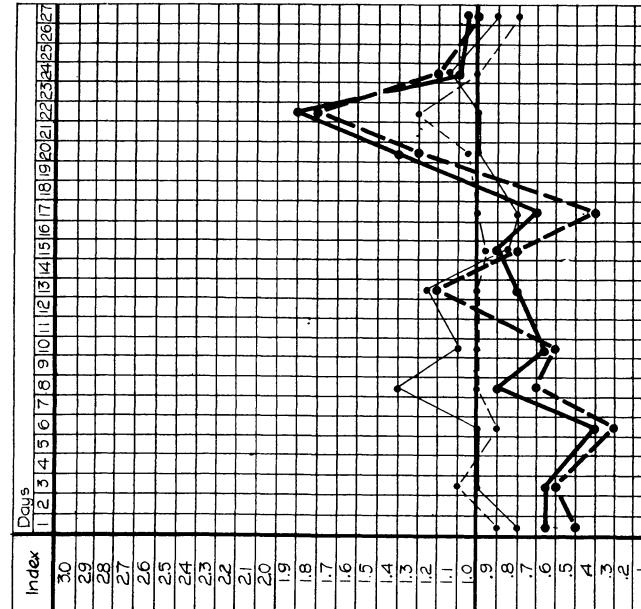


CHART 2.—Severe case of scarlet fever (adult man).
 Heavy solid line = Opsonic index with streptococcus from patient's throat.
 Heavy broken line = Opsonic index for homologous streptococcus with normal leukocytes.
 Fine broken line = Opsonic index with heterologous streptococcus.
 Fine solid line = Opsonic index with staphylococcus.
 Heavy solid line = Opsonic index with pneumococcus.

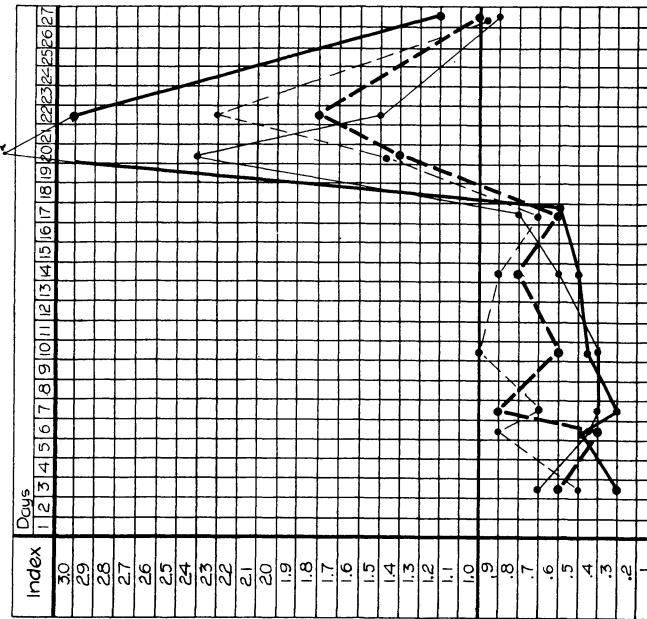


CHART 3.—Severe case of scarlet fever (adult man).
 Heavy broken line = Opsonic index for homologous streptococcus with normal leukocytes.
 Fine broken line = Opsonic index for homologous streptococcus with patient's leukocytes.
 Fine solid line = Phagocytic power of patient's leukocytes with respect to homologous streptococcus as compared with that of normal leukocytes under the influence of normal serum (cytophagic index).
 Heavy solid line = Phagocytic power of patient's whole blood with respect to homologous streptococcus as compared with that of normal blood (opsonocytophagic index).

came as active as normal. Nothing could be found to explain the low index. In the 30 experiments on this case, the destructive and phagocytic power of the serum and leukocytes followed the same general course.

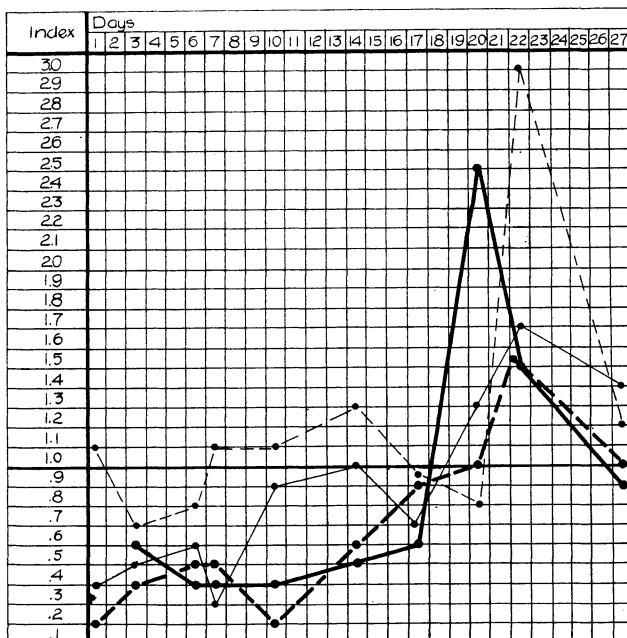


CHART 4.—Severe case of scarlet fever (adult man).

The Phagocytic Power of Patient's Leukocytes Compared with That of Normal Leukocytes under the Influence of Normal Serum (Cytophagic Index).

Heavy solid line = Homologous streptococcus index.

Fine solid line = Heterologous streptococcus index.

Heavy broken line = Pneumococcus index.

Fine broken line = Staphylococcus index.

In the eight severe cases the opsonic index was low to the invading streptococcus during the early stage, followed by an increase later on in the cases that recovered (Chart 2). The leukocytes also showed a diminished phagocytic activity early in the disease, with an increased activity as the patient's condition improved (Chart 3). These changes in the phagocytic activity were not specific for the

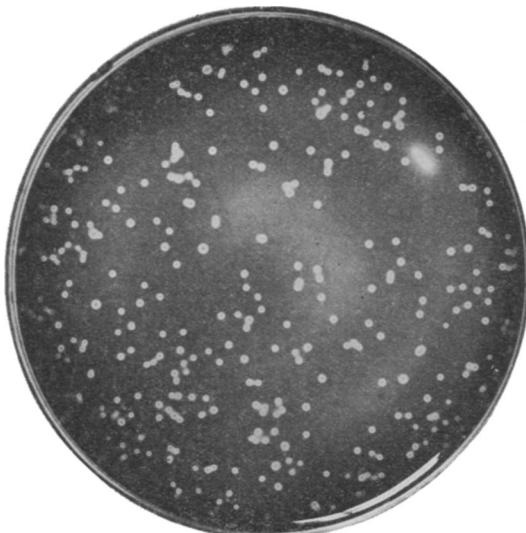


Plate 1.—Normal Serum and Normal Leukocytes.



Plate 2.—Patient's Serum and Normal Leukocytes.



Plate 3.—Normal Serum and Patient's Leukocytes.

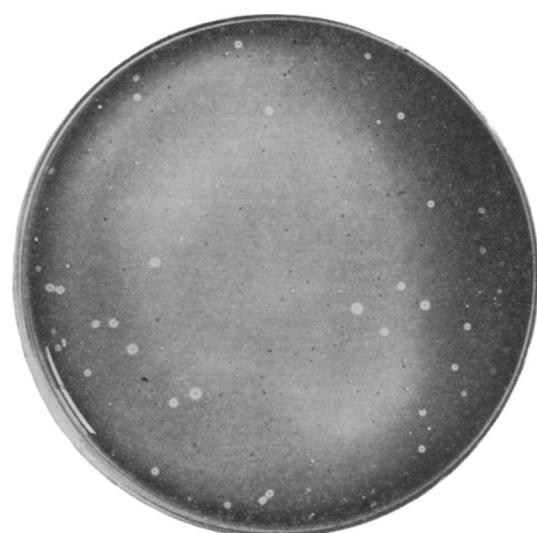


Plate 4.—Patient's Serum and Patient's Leukocytes.

On this particular day the opsonic power of the serum (opsonic index) and the phagocytic activity of the leukocytes (cytophagic index) were both above normal.

FIG. 1.—Photographs of blood-agar plates from mixtures of streptococci, leukocytes, and serum to show increased streptococcal power in case of severe scarlet fever with complications.

invading organism, but were demonstrable also for the pneumococcus and staphylococcus (Chart 4). In a large majority of the experiments, the destructive and phagocytic power of the serum and the leukocytes corresponded quite closely.

One septic case of scarlet fever showed a persistently low opsonic index and low phagocytic power of the leukocytes (cytophagic index) with correspondingly low streptococcidal power, although the patient was apparently well. A diffuse streptococcus phlegmon of the whole arm which developed in a few days perhaps explained

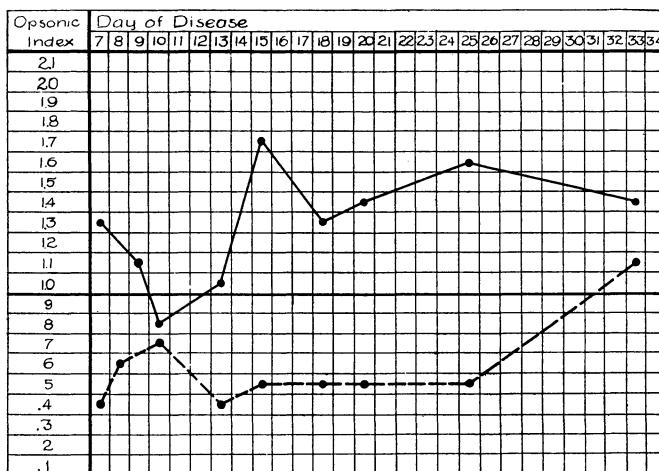


CHART 5.—Severe case of scarlet fever (adult woman).

Solid line = Streptococcus opsonic index with normal leukocytes.
 Broken line = Streptococcus opsonic index with patient's leukocytes.

this condition. On recovery from this infection of the arm, the opsonic index rose above normal, but the phagocytic power of the leukocytes remained low (cytophagic index) and the destructive power of the serum and leukocytes stayed below normal. A severe nephritis now set in. When this subsided, the opsonic index and the cytophagic index both increased, as did also the streptococcidal power of the serum and the leukocytes, but with the appearance of an abscess on the arm the destructive power of the whole blood again decreased, although the phagocytic activity remained quite high. The results of these two experiments are in accord with

the observations of Rosenow in cases of chronic endocarditis, in which he found that the amount of phagocytosis and the destructive power did not go together.

In almost every case, both of pneumonia and of scarlet fever, the opsonic index was the same whether normal or homologous leuko-

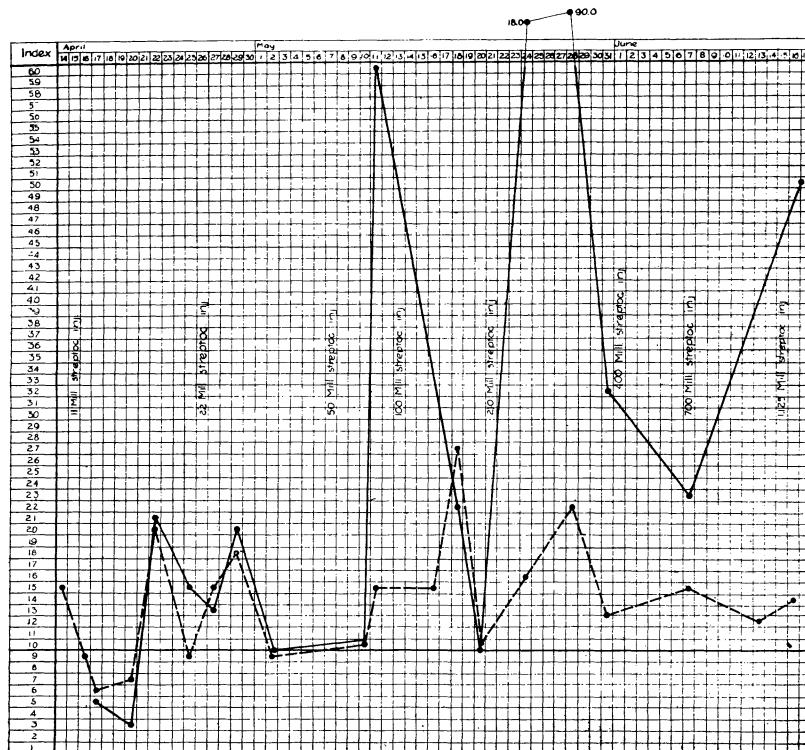


CHART 6.—Observations in the case of a boy, 7 years old, with chronic articular rheumatism, receiving injections of killed streptococcus cultures isolated from the tonsils.

Heavy broken line = Phagocytic power of the patient's leukocytes with respect to the streptococcus as compared with that of normal leukocytes under the influence of normal serum (cytrophagic index).

Heavy solid line = Streptococcal power of the patient's leukocytes as compared with that of normal leukocytes under the influence of normal serum.

cytes were employed (Chart 3). One scarlet fever patient, only, showed considerable variation in this respect (Chart 5).

The patient with chronic articular rheumatism (for the chance to study which I am indebted to Dr. John M. Dodson) showed an

increase in the amount of streptococcus opsonin and in the phagocytic activity of the leukocytes after each injection of the autogenous vaccine. Corresponding to this increase in opsonin and phagocytic activity there was generally also an increase in the destructive power of the serum and leukocytes. The streptococcal power of the patient's serum and leukocytes when combined also increased with the elevation of the total phagocytic power of the blood (opsono-cytophagic index). The details are given in Table 1 and Chart 6.

TABLE I.

A COMPARISON BETWEEN THE PHAGOCYTIC AND DESTRUCTIVE POWER FOR STREPTOCOCCUS OF THE SERUM AND LEUKOCYTES OF A PATIENT WITH CHRONIC RHEUMATISM AND OF NORMAL SERUM AND LEUKOCYTES.

DATE	Opsonic Power of Patient's Serum	Streptococci-dal Power of Patient's Serum and Normal Leukocytes as Compared with that of Normal Serum and Normal Leukocytes	Phagocytic Power of Patient's Leukocytes in Normal Serum as Compared with that of Normal Leukocytes in Normal Serum	Streptococci-dal Power of Patient's Leukocytes in Normal Serum as Compared with that of Normal Leukocytes in Normal Serum	Phagocytic Power of Patient's Blood as Compared with that of Normal Leukocytes in Normal Serum	Streptococci-dal Power of Patient's Blood as Compared with that of Normal Blood
Apr. 18	0.5	4.0	0.6	0.5	0.2	0.1
Apr. 20	0.5	0.3	0.7	0.4	0.6	0.5
Apr. 22	0.8	2.0	2.0	2.0	1.0	3.0
Apr. 25	0.9	6.0	0.8	1.5	0.92	2.0
Apr. 27	1.6	3.0	1.5	1.2	1.5	1.2
May 2	0.9	1.3	0.95	0.9	0.9	1.0
May 9	1.3	6.0	1.0	0.8	1.2	600.0
May 11	1.0	2.5	1.5	6.0	1.4	200.0
May 18	2.7	1.0	2.7	2.2	4.4	4.4
May 20	0.84	0.46	1.0	1.0	1.0	14.0
May 24	2.2	3.2	1.6	18.0	2.4	732.0
May 28	1.2	3.6	2.2	90.0	1.68	300.0
May 31	1.7	58.0	1.24	32.0	1.7	58.0
June 7	1.38	0.94	1.40	2.3	1.32	1.8
June 15	1.4	2.0	1.4	5.0	1.4	11.0

SUMMARY.

The observations here recorded indicate: (1) As a rule there is an increase in the phagocytic power of the leukocytes in mild cases of pneumonia. (2) In mild and in uncomplicated cases of scarlet fever, there is no change in the phagocytic activity of the leukocytes. (3) In severe cases of pneumonia and scarlet fever, the phagocytic power of the leukocytes is generally diminished during the height of the disease, but when the patient improves

it rises above normal, to which it returns during convalescence. (4) Although the opsonic power of the serum is specific, there is no such specificity in the phagocytic power of the leukocytes so far as my results indicate. (5) So far as pneumonia and scarlet fever are concerned, variation in the opsonic power of the serum and in the phagocytic activity of the leukocytes as a rule is accompanied by a corresponding variation in the pneumococcidal or streptococcidal power of the serum and the leukocytes.